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EVALUATION OF THE PROBABILITY OF USING TOTAL LYMPHOCYTE COUNT AS AN ALTERNATIVE TO CD4 COUNT IN INITIATION OF ART

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ABSTRACT
Measurement of $CD4^+$ T cell count in the diagnosis of HIV in a resource-constrain settings is often difficult due to high cost of equipments, reagents, and demand for trained personnel. There is need for relatively cost effective technique with good Prognosis for Human Immunodeficiency Virus (HIV) infection. Two hundred and fourty four (244) HIV seropositive (tests) and fifty (50) HIV seronegative individuals (controls) were analyzed for CD4 count and TLC. The result of the study was analyzed using SPSS 14, the mean $\pm$ SEM 930 $\pm$ 43 cells/µl (CD4 count) and 2026 $\pm$ 75 cells/mm^3 Total Lymphocyte Count (TLC) in control and 196 $\pm$ 8.0 cells/µl (CD4 count) and 1969 $\pm$ 66 cells/mm^3 (TLC) in Test group were obtained. There was significant difference between the values of CD4 count recorded in Test (196 $\pm$ 8.0 cells/µ) when compared with the value recorded in Control (930 $\pm$ 43 cells/µl) ($p<0.01$), but there was no significant difference in the values of TLC recorded in Test (1969 $\pm$ 66 cells/mm^3) when compared with the values recorded in the control (2026 $\pm$ 75 cells/mm^3) ($p<0.01$). This is due to the fact that CD4+T lymphocyte are specifically targeted by HIV which results in linear reduction in CD4 count not the whole population of T-lymphocytes and B-lymphocytes. A spearman's correlation study was done between the CD4 counts and TLC, indicated a slightly positive correlation ($r= 0.586$) among the test individuals and strong positive correlation in the control ($r = 0.739$). It could be concluded that total lymphocyte count can not be used alone as an alternative to CD4 count in initiating ART, but in conjunction with clinical sign and symptoms.
Keywords: Human immunodeficiency virus, CD4 count, Total lymphocyte count, and Antiretroviral therapy.

INTRODUCTION

According to the united nations program on HIV/AIDS (UNAIDS), more than 45 million people have been infected with the human immunodeficiency virus (HIV) since the first case was described in 1981. Over 90% of HIV infected people live in developing countries. The AIDS epidemic has resulted in a tremendous cost in terms of loss of lives and life-quality worldwide, especially in Africa, where 70% of deaths from HIV-1 infection have occurred (UNAID and WHO 2007). There is an emerging consensus that the HIV epidemic in the developing world requires treatment with antiretroviral drugs (Gange *et al.,* 2003). In well-resourced settings, the decision to initiate ART is based predominantly on the presence of HIV-related symptoms and on CD4+ T-cell count (Badri and Wood 2003). Absolute CD4+ T-cell counts and CD4+ percentages have constituted the mainstay criteria for monitoring progression in HIV-1 infected patients. CD4+ T-cell counts < 200 cell/mm3 or a CD4+ percentage < 20% is associated with an increased risk for *Pneumocystis jiroveci* pneumonia or infection with other opportunistic pathogens (Badri and Wood 2003).

Monitoring individuals with HIV infection/AIDS requires the use of expensive tools, which are not readily available in resource-limited settings (Akinola *et al.,* 2004). The identification of laboratory tests that help the clinician to predict progression is useful not only to monitor the patients' disease evolution but also to define the right time to initiate treatment (WHO 2005). In 2003, WHO recommended the use of absolute lymphocyte count as an alternative marker when a CD4+ cell count is not available or is not affordable. And recommended that a total lymphocyte count of ≤ 1200 lymphocytes/mm3 can be substituted for the CD4 count when the later is unavailable and HIV-related symptoms existed. One challenge for using TLC for predicting the disease stage is that it does not linearly decrease over time during HIV infection, but rather there is a period of stability, followed by a faster decay that precedes clinically-defined AIDS (Gange *et al.,* 2003).

This research was design to assess the level of CD4 cell count and total lymphocyte count in the HIV positive (tests) and HIV negative (controls), also to assess the relationship between CD4 cell count and TLC and to compare our findings with those obtainable in other part of the world.

MATERIALS AND METHODS

The study was conducted at laboratory department of Infectious Diseases Hospital (I.D.H.) Kano. Two hundred and fourty four (244) newly diagnosed HIV seropositive (tests) individuals (112 males and 132 females) distributed among 4 age groups (20-30: 89, 31-40: 96, 41-50: 43, 51-60: 16) and fifty (50) apparently healthy

HIV seronegative (controls) individuals (21 male and 39 females) distributed among 4 age groups (20-30: 34, 31-40: 10, 41-50: 4, 51-60: 2) were enrolled in this study. A 5ml whole blood sample from each patient was collected in EDTA vacutainer tubes in the morning between 8:00am to 10:00am.

The Total CD4+ T Lymphocytes was determined using an automated flow cytometer (Partec Cyflow Counter 060727120, Germany). The blood samples were processed and analyzed within six hours from the time samples were collected. And the total lymphocyte were obtained from Full Blood Count results, using an Automated Hematology Analyzer (Sysmex KX-21N, Sysmex Corporation. Results obtained were analyzed using SPSS 14.0 for windows.

RESULTS

The results obtained in this study are expressed as mean $\pm$ SEM (Standard error of mean) for the control and the test individuals respectively. These are presented in Tables 1 - 4 and Figures 1 and 2.

Table 1: CD4 and Total Lymphocyte Count (TLC) in Test and control

Sample	n	CD4 Count	Total Lymphocytes
Control	50	930 $\pm$ 43	2026 $\pm$ 75
Test	244	196 $\pm$ 8.0	1969 $\pm$ 66

It can be seen that there was significant difference between the values of CD4 count recorded in Test (196 $\pm$ 8.0 cells/µ) when compared with the value recorded in Control (930 $\pm$ 43 cells/µl) ($p<0.01$). lBut there was no significant difference in the values of TLC recorded in Test (1969 $\pm$ 66 cells/mm^3) when compared with the values recorded in the control (2026 $\pm$ 75 cells/mm^3) ($p<0.01$).

Table 2: CD4 count and Total Lymphocyte Count (TLC) in Females Test and Control subjects

Sample	n	CD4 Count	Total Lymphocytes
Control	29	1031 $\pm$ 60	2165 $\pm$ 99
Test	132	219 $\pm$ 12	1760 $\pm$ 70

Table 3: CD4 count and Total Lymphocyte Count (TLC) in Males Test and Control subjects

Sample	n	CD4 Count	Total Lymphocytes
Control	21	790 $\pm$ 46	1833 $\pm$ 104
Test	112	170 $\pm$ 10	1743 $\pm$ 96

Table 4: Spearman's Correlation Coefficient (r) of CD4 Count and TLC in Test and Control

	HIV Seronegative		HIV Seropositive	
	n	r	n	r
Male & Female	50	0.739	244	0.586
Male	21	0.764	112	0.611
Female	39	0.693	132	0.569
20-30 yrs	34	0.747	89	0.593
31-40 yrs	10	0.839	96	0.625
41-50 yrs	4	0.400	43	0.464
51-60 yrs	2	1.0	16	0.696

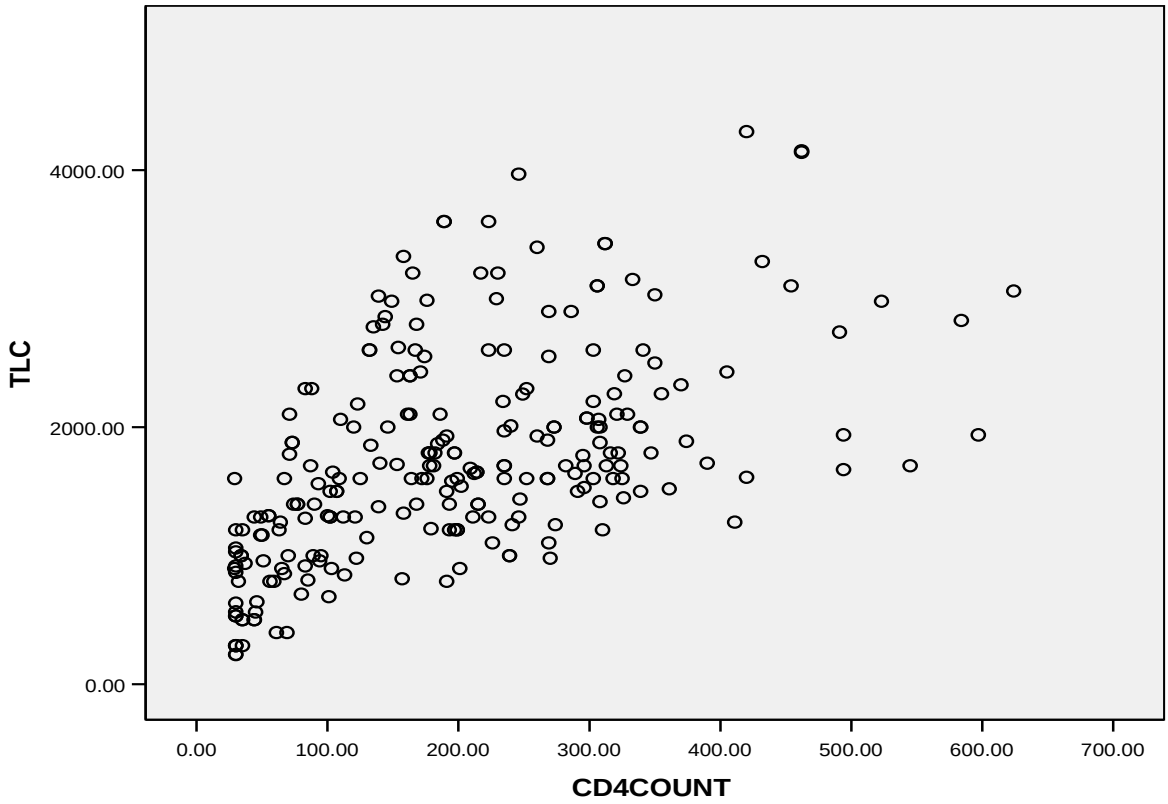

Figure 1: Scatter Graph of CD4 Count and TLC in Test subjects.
A scatter graph of spearman's correlation study of CD4 count and TLC in test subjects indicating a slightly positive correlation (r= 0.586).

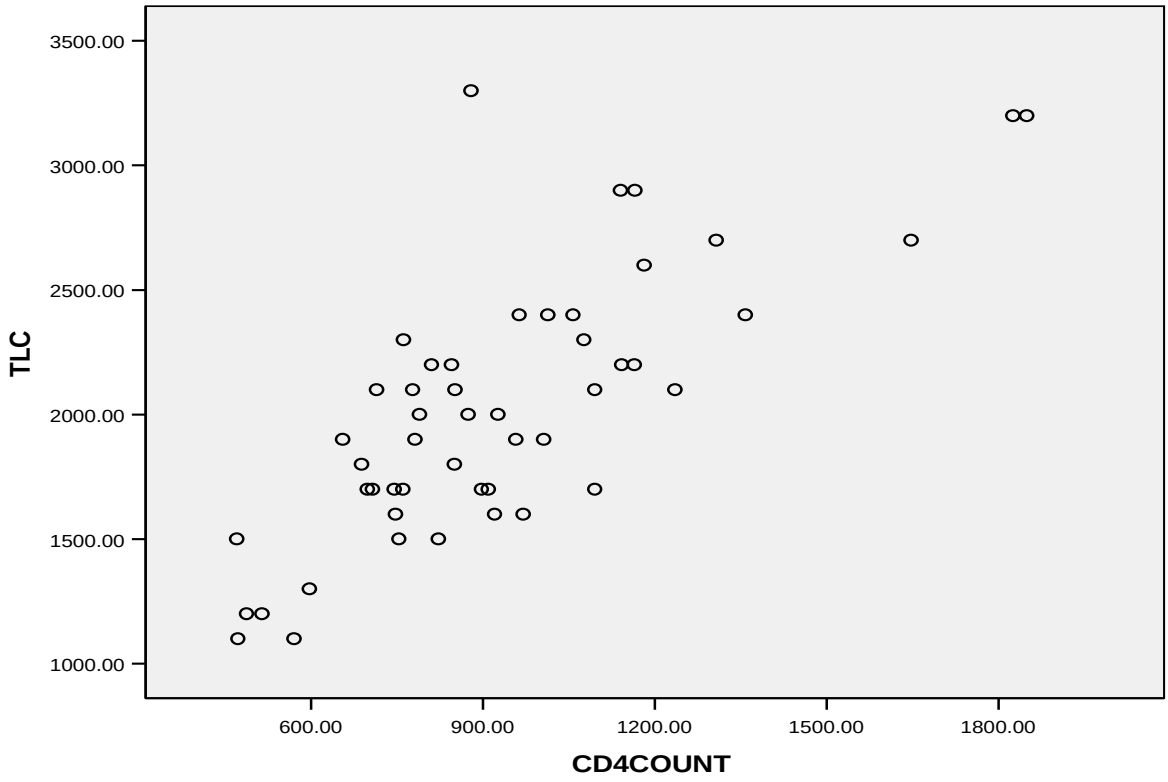

Figure 2: Scatter Graph of CD4 Count and TLC in Control subjects.
A scatter graph of spearman's correlation study of CD4 count and TLC in control subjects indicating strong positive correlation (r = 0.739).

DISCUSSION

From our result, we recorded a mean of 930 $\pm$ 43 cells/µl (CD4 count), 2026 $\pm$ 75 cells/mm^3 (TLC) in control and 196 $\pm$ 8.0 cells/µl (CD4 count) and 1969 $\pm$ 66 cells/mm^3 (TLC) in Test group. It can be seen that there was significant difference between the values of CD4 count recorded in Test (196 $\pm$ 8.0 cells/µ) when compared with the value recorded in Control (930 $\pm$ 43 cells/µl) ($p<0.01$). But there was no significant difference in the values of TLC values recorded in Test (1969 $\pm$ 66 cells/mm^3) when compared with the values recorded in the control (2026 $\pm$ 75 cells/mm^3) ($p<0.01$). This was due to the facts that CD4+T lymphocyte are specifically targeted by HIV which results in linear reduction in CD4 count. A spearman's correlation study was done between the CD4 counts and TLC, there was a slightly positive correlation ($r= 0.586$) in the test and strong positive correlation in control ($r = 0.739$).

Considering the above resuls this work agrees with the findings of Gange *et al.,* (2003), Johnson *et al.,* (2009) and Akinola *et al.*, (2004): Gange *et al* (2003) reported that total lymphocyte count does not linearly decrease over time during HIV infection, but rather there is period of stability followed by a faster decay that precedes clinically defined AIDS. Johnson *et a.l,* (2009) reported that low absolute lymphocyte count does not correlate with the severe immune suppression based on CD4 cell count, it also does not improve ability to identify children in need of ART. Akinola *et al.*, (2004) and Vand Der Ryst *et al.*, (1998) found that TLC is not a good predictor of the CD4+ T-cell count.

This work disagree with the work of Fournier and Sosenko (1992) and Blatt *et al.*, (1993). Fournier and sosenko (1992) stated that total lymphocyte count has a clinical utility as a predictor of AIDS stage. Blatt *et al.*, (1993) reported that TLC was a useful indicator of significant immune suppression.

COCLUSION

Total lymphocyte count could not be used alone as an alternative to CD4 count in initiating ART, but in conjunction with clinical sign and symptoms.

RECOMMENDATION

It could therefore be recommended that larger number of patients be used and other factors that affect immune system like nutritional status and presence or absence of coinfection be considered.

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